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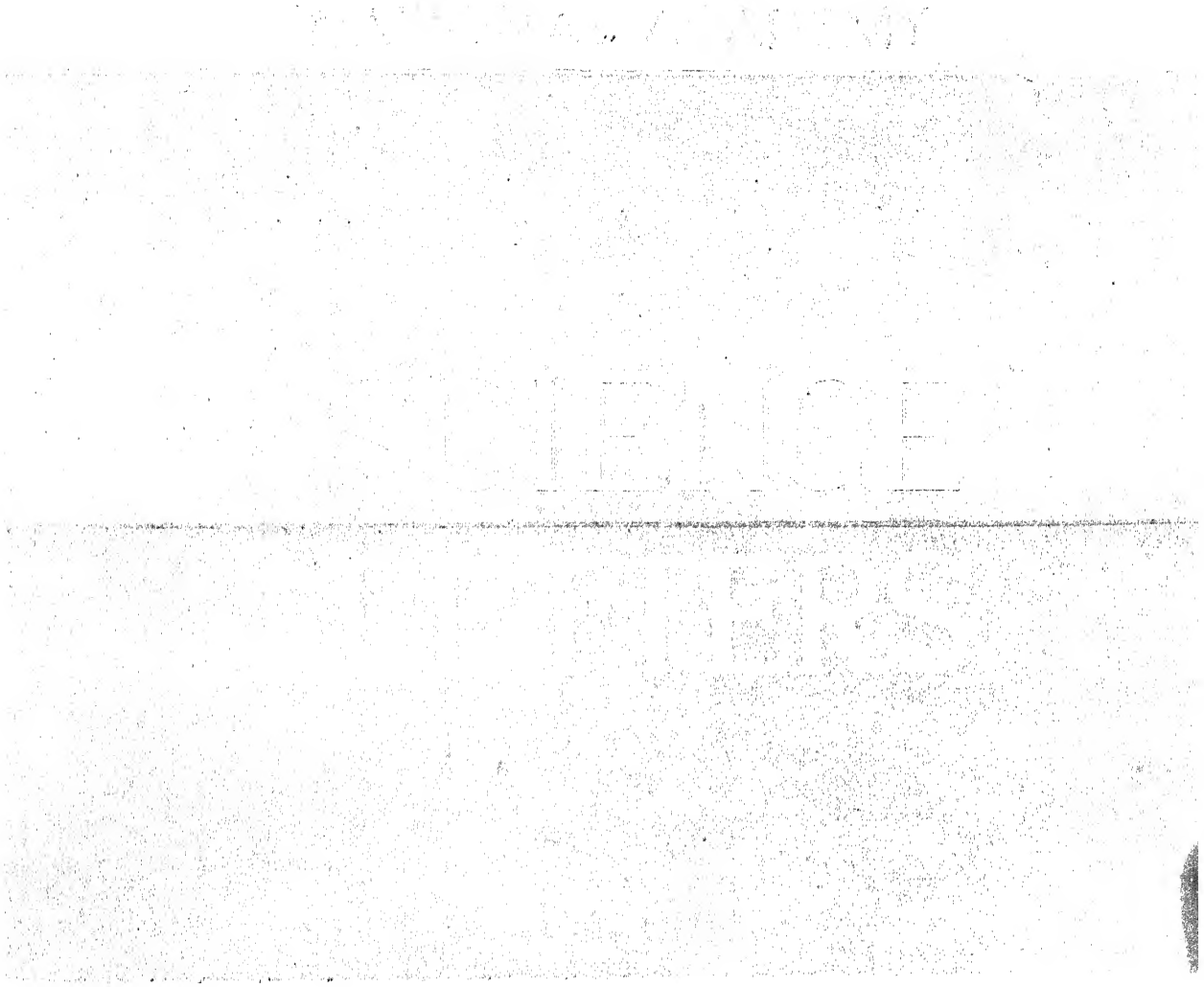

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Fungitoxic properties of some seedling extracts

(fungitoxicity/*Botrytis cinera*/*Colletotrichum gleoesporioides*)

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ABSTRACT Seedling extracts of 40 taxa belonging to 22 families were tested for their antifungal activity against *Botrytis cinera* Pers. and *Colletotrichum gleoesporioides* Penzig. The extracts of *Abrus precatorius*, *Brassica campestris*, *Carum copticum* and *Raphanus sativus* exhibited 100% inhibition of spore germination of both the test fungi while others were ineffective or partially effective.

The tender seedling stage of higher plants appear most vulnerable to infection by pathogens. But a large number of plants escape infection there by indicating the presence of some fungitoxic substance (s). Some seedling extracts were earlier reported[1] to possess strong fungistatic properties. The present communication deals with the screening of some other seedlings for their fungitoxic activity.

The antifungal activity of the seedling extracts were tested by the slide-germination-technique[2]. In order to obtain the seedling extracts, seeds of various species of higher plants were allowed to germinate on moist filter-paper in presterilized petri-dishes for two to three weeks. Entire seedlings were crushed thoroughly to extract the juice, which was centrifuged at 3000 rpm for 5 min. The fungitoxicity of clear supernatant was tested against *Botrytis cinera* Pers. and *Colletotrichum gleoesporioides* Penzig and the fungitoxic activity was measured in terms of percentage inhibition of spore germination[1].

TABLE 1

Fungitoxic Activity of some plant seedlings

S. No.	Botanical Name	Local Name	Family	% inhibition of spore germination			
				Botrytis cinerea		*Colletotrichum gleoesporioides*	
				Crude	Diluted (1 : 5)	Crude	Diluted (1 : 5)
1.	*Abrus precatorius* Linn.	Ghumachi	Fabaceae	100	100	100	100
2.	*Abutilon indicum* (L.) Sweet	Kanghi	Malvaceae	*	*	21.79	10.25
3.	*Ageratum conyzoides* Linn.	Nilam	Asteraceae	45.53	40.00	60.25	48.71
4.	*Allium cepa* Linn.	Pyaz	Liliaceae	75.00	68.00	84.81	59.62
5.	*Anethum graveolens* Linn.	Sowa	Apiaceae	72.00	46.67	55.12	28.20
6.	*Arachis hypogaea* Linn	Moongfali	Fabaceae	20.51	10.25	5.95	4.73
7.	*Argemone mexicana* Linn.	Bhar-bhar	Papaveraceae	53.33	42.67	46.15	34.61

Table 1 Contd.

S. No.	Botanical Name	Local Name	Family	% inhibition of spore germinaton			
				Botrytis cinerea		*Collectorichum gleoesporioides*	
				Crude	Diluted (1 : 5)	Crude	Diluted (1 : 5)
8.	*Azaairachta indica* A. Juss	Neem	Maliaceae	80.75	65.38	75.00	53.57
9.	*Brassica oleracea* var *capitata* Linn.	Patgobhi	Brassicaceae	75.64	46.15	45.12	41.46
10.	*Brassica oleracea* var. *caulorapa* Linn.	Ganthgobhi	Brassicaceae	41.62	38.45	48.17	30.48
11.	*Brassica campestris* Linn.	Sarson	Brassicaceae	100	100	100	100
12.	*Carica papoya* Linn.	Papita	Caricaceae	*	*	5.00	22.50
13.	*Carum copticum* Hiern	Ajowain	Apiaceae	100	100	100	100
14.	*Cassia occidentalis* Linn.	Bara chakwar	Caesalpini-aceae	1.31	11.84	10.00	15.00
15.	*Cassia tora* Linn.	Chhota chakwar	Caesalpini-aceae	14.47	10.52	10.00	*
16.	*Cleome viscosa* Linn.	Hur-hur	Capparidaceae	*	*	11.53	10 25
17.	*Cicer arietinnm* Linn.	Chana	Fabaceae	6.94	1.38	11.49	22 78
18.	*Coriandrum sativum* Linn.	Dhania	Apiaceae	66-67	48.00	62.82	46.15
19.	*Cucumis sativus* Linn.	Khira	Cucurbitaceae	*	*	*	*
20.	*Daucus carota* Linn.	Gazar	Apiaceae	13.33	5 33	36.90	21.42
21.	*Dolichos lablab* Linn.	Sem	Fabaceae	6.94	27.78	25.31	17 72
22.	*Foeniculum vulgare* Mill.	Sauni	Apiaceae	56 00	46.67	74.16	52.56
23.	*Gossypium arboreum*	Kapas	Malvaceae	11.53	7.69	8.04	6.89
24.	*Ipomoea fistulosa* Mart.	Behaya	Convolvula-ceae	13.33	10.66	38.76	23.08
25.	*Lagenaria siceraria* Ser. Mart	Lauki	Cucurbitaceae	72.00	53.84	59 76	51.72
26.	*Linum usitatissimum* Linn.	Alse	Linaceae	24.00	20 00	20.23	27.38
27.	*Luffa cylindrica* (L) Roem.	Nenuwa	Cucurbitaceae	*	*	*	*
28.	*Lycopersicum esculentum* Mill.	Tamatar	Solanaceae	*	*	*	*
29.	*Mangifera indica* Linn.	Aam	Anacardiaceae	100	100	82.14	53.57
30.	*Momordica charantia* Linn.	Karela	Cucurbitaceae	77 46	44.00	88.09	53.57
31.	*Moringa oleifera* Lamk.	Sahajan	Moringnaceae	29.48	23.08	43.79	39 08
32.	*Ocimum sanctum* Linn.	Tulsi	Lamiaceae	50.00	48.71	70.11	67.81
33.	*Ocimum canum* Sims.	Ban Tulsi	Lamiaceae	100	100	100	100
34.	*Oxalis corniculata* Linn.	Tin patia	Oxalidaceae	*	*	*	*
35.	*Raphanus sativus* Linn.	Mooli	Brassicaceae	100	100	100	100
36.	*Ricinus communis* Linn.	Rendi	Euphorbiaceae	46.15	43.58	28.73	27.58
37.	*Syzygium cumini* (L.) Skeels	Jamun	Myrtaceae	74 36	56.00	41.67	33.33
38.	*Tamarindus indica* Linn.	Imali	Caesalpini-aceae	39.74	26,92	38.01	27.38
39.	*Trigonella-foenum* Linn.	Menthi	Papilionaceae	32.00	25.33	41.67	38 09
40.	*Zizyphus mauritianum* Lamk.	Ber	Rhamaceae	3.48	1.28	2.38	*

* = Fungus growth stimulated

Out of 40 taxa screened the seedling extracts of *Abrus precatorius*, *Brassica campestris*, *Carum copticum* and *Raphanus sativus* exhibited 100% inhibition of spore germination of both the test fungi. However, extracts of *Allium cepa*, *Azadirachta indica*, *Coriandrum sativum* and *Momordica charantia* were partially active. The extracts of *Cucumis sativus*, *Luffa cylindrica*, *Lycopersicum esculentum*, *Oxalis corniculata* stimulated the spore germination of both the test fungi while that of *Abutilon indicum*, *Carica papaya*, *Cleome viscosa* and *Zizyphus mauritianum* stimulated spore germination of one of the test fungi (Table 1).

Thanks are due to Head, Botany Department, Gorakhpur University for providing necessary facilities, to ICAR (New Delhi) and CSIR (New Delhi) for financial assistance.

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Effects of SO_2 exposure on flowering, yield and carbohydrate contents of *Vigna sinensis*

(SO_2 pollution/flowering/yield/carbohydrates/*Vigna sinensis*)

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ABSTRACT Effect of 0.12, 0.25 and 0.5 ppm SO_2 fumigations has been observed on flowering, pod maturation, yield and carbohydrate contents of two cultivars of *Vigna sinensis*, Type-2 and Pusa Barsati The flowering and first pod maturation were advanced by 1-5 days as a result of SO_2 fumigation. There was a decrease in the number of pods per plant, length of the pod, number of seeds per pod, 100-seed weight, seed yield per plant, yield and harvest index. Carbohydrate contents of the stem, leaf and seed were also adversely affected. Of the two cultivars, Type-2 was more susceptible to SO_2 pollution.

Sulphur dioxide is an important air pollutant causing damage to plants growing in the vicinity of industrial establishments. The present study was undertaken to assess the effects of SO_2 on yield and carbohydrate contents of two cultivars, Type-2 and Pusa Barsati of *Vigna sinensis* (Lobiya).

Seeds of *Vigna sinensis* (L.) Savi ex Hassk cv. Type–2 and Pusa Barsati, o'tained from I.A.R.I., New Delhi, were sown in experimental plots with line to line distance of 15 cm and plant to plant distance of 10 cm in Kharif season of 1984. Normal agronomic practices were followed. The plants were fumigated with three concentrations of SO_2, 0.12, 0.25 and 0.5 ppm from 10 days after sowing to pod maturation. The fumigation was done for 6 hours each day (10 a.m. to 4 p.m.) for six days a week (no fumigation was done on sundays), in 1 m^3 polythene chambers, supported on aluminium frames. SO_2 was generated by passing a continuous current of air through 1% aquous solution of sodium metabisulphite and introduced into the fumigation chamber through perforated alkathene tubes. The concentration of SO_2 was monitered at hourly intervals. A known volume of air from the fumigation chamber containing SO_2 was passed through 0.1 M solution of sodium tetrachloromercurate which was assayed for SO_2 concentration following West and Gaeke[1]. A control was run in identical conditions without SO_2 fumigation. Carbohydrate contents were determined following Yemm and Willis[2].

Exposure to SO_2 advanced the flowering by 2–5 days in cultivar Type–2 and 1–4 days in Pusa Barsati. Pod maturation was also advanced but the effect was statistically significant in Pusa Barsati only. Singh and Rao[3] have also reported advance of flowering in *Cicer arietinum* as a result of SO_2 pollution.

The unmber of pods per plant, length of pod, number of seeds per pod, 100 seed weight and yield per plant were adversely affected by SO_2 exposures (Table 1). The effects became more pronounced with the increasing SO_2 concentrations. There was 7, 12 and 25 per cent reduction in the number of seeds per pod in Type–2 and 7, 18 and 25 per cent in Pusa Barsati in 0.12, 0.25 and 0.5 ppm

TABLE 1

Effects of SO_2 on the yield parameters of *Vigna sinensis* cultivars.

Culti-var	Paremeter/ Treatment	Days to first flowering	Days to first pod maturation	Number of pods/ plant	Length of the pod (cm)	Number of seeds/pod	Seed yield/ plant (g)	100-seed weight (g)	Yield (g)	Harvest index (%)
Type-2	Control	75.0±0.54	90.0±1.00	48.0±4.38	16.87±1.30	16.75±1.57	85.700±5.56	10.650±1.01	150.100±8.64	56.86±2.24
	0.12 ppm SO_2	73.2±0.44**	90.0±0.54+	40.5±3.67*	15.75±1.14+	15.50±1.09+	65.010±4.87**	10.050±0.95+	130.810±7.56**	49.69±2.01**
	0.25 ppm SO_2	72.0±1.00**	89.0±0.44+	35.6±4.07**	15.62±1.07+	14.75±1.21+	49.200±3.19**	9.180±0.89+	108 200±8.24**	45.47±1.87**
	0.5 ppm SO_2	70.0±0.54**	89.0±0.44+	31.2±3.12**	14.37±1.09*	12.50±1.00**	36.250±3.15**	8.540±0.70**	95.300±6 23**	38.03±1.63**
Pusa	Control	47.0±0.44	65.2±0.54	26.5±2.21	20.20±1.93	16.3±1.37	44.000±2.54	10.250±1.00	73.800±4.74	59.62±2.14
Barsati	0.12 ppm SO_2	46.0±0.44**	64.8±0.44+	22.2±1.83**	19.80±1.57+	15 30±1.51+	34.500±2.56**	9.820±0.91+	62 700±4.21**	55.02±1.79**
	0.25 ppm SO_2	44 6±0.54**	64.0±0.44**	18.5±1.89**	19.00±1.31+	13.50±1.14**	28.600±2.17**	9.050±0.84+	56.000±3 87**	51.07±1.83**
	0.5 ppm SO_2	43.3±0.44**	63.2±0.54**	16.0±2.01**	18.50±1.14+	11.50±1.09**	23.500±2.07**	8.140±0.72**	49.600±4.01**	47.37±1.07**

Values are in mean ± S. D. ; Significance of difference from control : *$p < 0.05$, **$p < 0.01$, + non-significant.

SO_2, respectively. The seed yield per plant was significantly reduced even at 0.12 ppm SO_2 in both cultivars. Consequently there was a decrease in yield and harvest index.

Sprugel *et al.*[4] reported 5–48% reduction in yield of soybeans exposed to 0.09–0.79 ppm SO_2. They also observed a mean decrease in seed weight and number of seeds per plant. Significant reductions in yield have been reported as a result of SO_2 pollution[3,5] in many cereal and legume crops. SO_2 induced inhibition of net photosynthesis has been observed in many crop plants *e.g. Medicago sativa*[6] and *Pisum sativum*[7] and this eventually leads to decrease in seed weight, number of seeds per pod and number of pods per plant and hence reduction in the total yield.

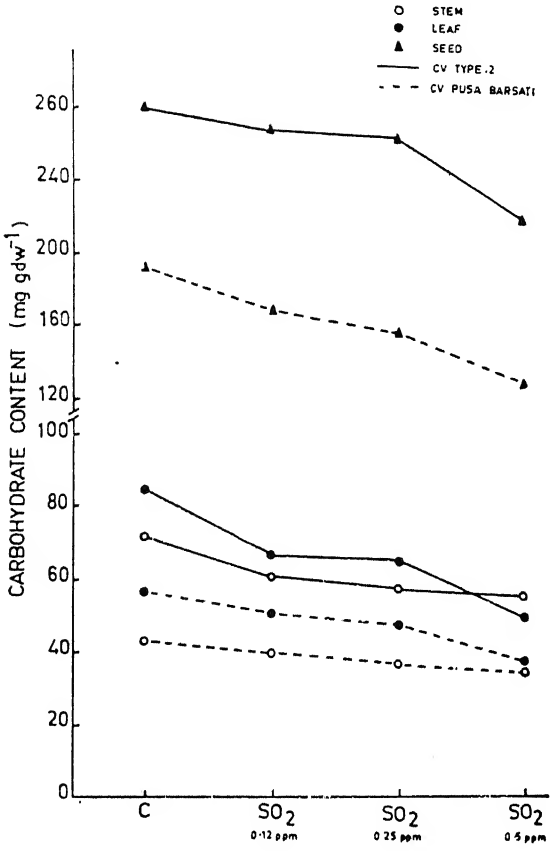

Fig. 1. Total carbohydrate content in stem, leaves and seeds of *Vigna sinens s* cultivars, Type-2 and Pusa Barsati fumigated with three concentrations (0.12, 0.25 and 0.5 ppm) of SO_2 besides unfumigated as control.

There was a decrease in carbohydrate contents of stem, leaf and seed in all concentrations of SO_2. While the percentage decrease in stem and leaf carbohydrate was higher in cultivar Type-2, in seeds it was Pusa Barsati (Fig. 1). Koziol and Jordan[8] reported that the reduction in carbohydrate content in SO_2 pollution may be due to increased respiration and decreased CO_2 fixation. In response to SO_2 toxicity, material and chemical energy was diverted from sites of growth and storage to sites where repair was needed. Such a diversion of material hamper the net productivity and hence carbohydrate content of SO_2-treated plants.

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A redox synthesis of polyacrylamide and determination of its molecular dimensions from viscosity data

(redox synthesis/polyacrylamide/molecular dimensions/viscosity)

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ABSTRACT Polyacrylamide has been synthesized by redox initiated polymerization of acrylamide in aqueous medium, using MnO_4^- - $H_2C_2O_4$ couple. The viscosity average molecular weights and some molecular dimensions of the prepared polymer samples have also been estimated.

The unperturbed dimensions of polymers have either been obtained from the intrinsic viscosity–molecular weight data in theta solvent or by extrapolation of the required data in a good solvent, according to the relation proposed by Stockmayer and Fixman[1]. Such dimensions have already been reported in case of chlorinated stereo-regular polybutadiene[2], polystyrene[3], poly (*p*-chlorostyrene)[4], poly (*p*-methoxystyrene)[5] and polyethylene terephthalate[6]. It was considered desirable to investigate the magnitude of molecular weight and some other parameters of polyacrylamide,synthesized by MnO_4^-–$H_2C_2O_4$ redox initiated polymerization of acrylamide. The present communication is an outcome of such an attempt.

Acrylamide (HPCI, Japan) was purified by the usual procedure[7] and other reagents of (B.D.H.) A. R. grade were used as such.

The polymerization of acrylamide was carried out under an inert atmosphere of nitrogen with $KMnO_4$–$H_2C_2O_4$ redox couple in aqueous medium. 50 ml of 1M acrylamide and 30 ml of 1M oxalic acid solutions were taken in a reaction vessel and maintained in a thermostat at 30°C. The contents of the vessel were freed from dissolved O_2 by passing a stream of oxygen-free N_2 first at a high rate and then at a rate of one bubble per s. After an hour, polymerization was initiated by introducing 4 ml of 0.1 M $KMnO_4$ solution.

The polymer was isolated after 5 min of permanganate addition by slowly pouring the reaction mixture into an excess of ice-cooled methanol. The mixture was allowed to stand overnight at 0°C and the white and rigid polyacrylamide crop was then filtered and washed with methanol. This (sample A) was then dried to a constant weight in a vacuum oven at 60 ± 1°C. Similar procedure was adopted to obtain other polymer samples B, C, D, E and F after the expiry of 10, 20, 30, 40 and 50 min respectively.

The coefficient of viscosity of aqueous solutions of each polyacrylamide sample was determined with an Ubbelohde viscometer at 30 ± 0.1°C.

Intrinsic viscosities $[\eta]$ of different polymer samples were obtained by extrapolating the straight lines obtained by plotting the graph between η_{sp}/C *vs* C (conc. in g/dl of solution) at infinite dilution. The $[\eta]$ values, thus obtained, for each polymer sample have been utilized to estimate the molecular weight of polacrylamide by means[8] of eqn.

$$[\eta] = 68 \times 10^{-5} M_v^{0.66} \quad (1)$$

The results are summarized in Table 1.

TABLE 1

Yield, intrinsic viscosity and Mol. wt. of polyacrylamide samples

Polymer sample	Polymer ppted. after time (min)	Yield (g)	$[\eta]$ (dl g^{-1})	Mol. wt $\times 10^{-5}$
A	5	2.45	1.11	0.74
B	10	2.62	1.28	0.92
C	20	2.75	1.18	0.81
D	30	3.05	1.01	0.64
E	40	3.04	1.05	0.68
F	50	3.05	1.13	0.76

It is clear from Table 1 that the yield of polyacrylamide tends to increase slowly upto 30 min to become practically constant onwards. The average molecular weight of the samples appear to be approximately constant ($\sim 1\times10^5$). Such a trend is in agreement with the characteristics of a chain polymerization reaction.

The intrinsic viscosity $[\eta]$ and viscosity average mol. wt. of a polymer ($\approx$ weight average mol. wt.[9], M_w) are connected by means of the equation proposed by Stockmayer and Fixman[1] as

$$[\eta] = KM_w^{1/2} + 0.51\, \varphi BM_w \quad (2)$$

where K is a parameter, characteristic of the polymer, B is polymer-solvent interaction and φ is a universal viscosity constant assumed to be same (2.5×10^{21}) for the polymers[10].

A plot of $[\eta] / M_w^{1/2}$ against $M_w^{1/2}$ appears to yield a straight line according to eqn (2). The values of K and B have been obtained from the intercept and slope of this graph.

Now K is related to the root mean square gyration radius $\bar{r}_0^2$ by the following eqn.

$$K = \phi \left(\frac{\bar{r}_0^2}{M_w}\right)^{3/2} \quad (3)$$

From the knowledge of the values of K and ϕ, $\left(\frac{\bar{r}_0^2}{M_w}\right)^{1/2}$ was thus calculated by means of eqn (3). The final results are summarized as follows

Mol. wt.	$K \times 10^5$	$B \times 10^{27}$	$\left(\frac{\bar{r}_0^2}{M_{w\cdot o}}\right)^{1/2} \times 10^8$
$\sim 10^5$	275	3.9	1.0320

The values of K and $\left(\frac{r_0^2}{M_w}\right)^{1/2}$ appear to be in accordance with those reported by Stockmayer and Kurata[11]. B also shows the same order as reported by Meyerhoff and Shimotsuma[6].

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Analysis of pyrazolone derivatives (antipyrine derivatives) using potassium hexacyanoferrate(III) and N-chlorosuccinimide in acidic medium

(pyrazolone/antipyreties/potassium hexacyanoferrate(III)/N-chlorosuccinimide)

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ABSTRACT Micro-analysis of antipyrine derivatives with potassium hexacyanoferrate(III) (PHC) and N-cholorosuccinimide (NCS) reagent in acidic medium is reported. Aliquots containing 1–15 mg of the samples were allowed to react either with excess of 0.1N PHC in 10 ml of 8M sulphuric acid and 5 ml of 3% zinc sulphate solution at 35°C in thermostatically controlled water-bath for 25 min or with 0.01N NCS in 10 ml of 4M HCl at room temperature for 15 min and the unconsumed reagents were determined iodometrically. In case of pure compounds as well as pharmaceutical preparations the results are within 0 ± 1% error.

Antipyrine and its derivatives *e.g.* Melubrin, Dipyrone (Analgin) and Amidopyrine (Pyramidon) are well known analgesic and antipyretic drugs. Antipyrine and its derivatives were estimated by colorimetric[1-4], complexometric[5,6], conductometric[7], potentiometric[8], non-aqueous titrimetric[9], titrimetric[10-12] and polarographic[13] procedures.

PHC and NCS have been applied for the determination of many organic compounds[14,15]. In the present communication, a simple and convenient procedure is described for the determination of antipyrine and its derivatives with PHC and NCS in acidic medium. PHC is superior to many other reagents because of its being used as primary standard[16], high equivalent mass, and extremely stable in acidic and basic media. NCS has high potentiality of acting as chlorinating and oxidising agent. The reagent is stable and gives quick reactions.

Solutions of 0.1N PHC (A.R., B.D.H.) and 0.1N sodium thiosulphate (A.R., B D H) were prepared in distilled water while 0.01N NCS (Reidel-DeHaenag, Germany) solution was prepared in warm water. Solutions of potassium iodide (10%), sulphuric acid (8M), hydrochloric acid (4M), zinc sulphate (3%) and starch (2%) were also prepared.

Sample solutions (1 mg/ml) were prepard by dissolving accurately weighed 50 mg compound in 50 ml volumetric flask in distilled water.

Solutions of Analgin, Novalgin and Esgipyrine tablets were prepared by dissolving well powdered tablets equivalent to 50 mg of pure sample in minimum amount of distilled water. The solution was filtered and filtrate was made upto the mark in 50 ml volumetric flask by distilled water.

Solution of Quinorsol injection was prepared by dissolving a certain volume of injection equivalent to 50 mg of pure sample in distilled water in a 50 ml volumetric flask.

Aliquots containing 1–15 mg of sample were taken in 100 ml erlenmeyer flasks followed by the addition of 8 ml of 0.1 N PHC reagent, 10 ml of 8 M

sulphuric acid and 5 ml of 3% zinc sulphate. The reaction mixture was shaken thoroughly and allowed to react at 35°C in thermostatically controlled water-bath for 25 min. In case of NCS, 10 ml of 0.01N NCS and 10 ml of 4M hydrochloric acid were added and contents were allowed to react at room temperature for 15 min. Unconsumed reagents were determined iodometrically by titrating against 0.1N and 0.01N sodium thiosulphate solution for PHC and NCS respectively. A blank was also run in both the cases. The procedure was also applied for the determination of these compounds in pharmaceutical preparations. Tablet excipients *e.g.* talcum powder, starch, gum, magnesium stearate etc. do not interfere. The accuracy of the results is within ±1%.

The stoichiometry of the reaction was established and the recovery of the sample was calculated by the following eqn.

$$\text{Mass of sample (in mg)} = \frac{M.N(B-S)}{n}$$

where, B is volume of sodium thiosulphate for the blank experiment, S is volume of sodium thiosulphate for the actual experiment, M is molecular mass of the sample, N is the molarity of sodium thiosulphate and n is number of moles of reagent/mole of sample.

The recommended procedures were successfully applied for the estimation of 2,3-dimethyl-1-phenyl pyrazol-5-one (Antipyrine), Melubrin. N-Methyl Melubrin (Analgin) and dimethyl amino antipyrine

TABLE 1

Determination of pyrazolone derivatives with PHC and NCS in acidic medium.

Sample	PHC Method*				NCS Method*			
	Reaction time (min)	Molecularity	Amount obtained by calculation	SD/mg	Reaction time (min)	Molecularity	Amount obtained by calculation	SD/mg
1. 2,3-Dimethyl-1-phenyl pyrazol-5-one (Pure) (Antipyrine)	15	6	99.95	0.0253	10	6	99.75	0.0234
a. Quinorsol injection[1]	15	6	100.17	0.0111	10	6	99.80	0.0380
2. Melubrin (Pure)	20	6	100.37	0.0113	10	5	100.28	0.0178
3. Methyl Melubrin (Pure) (Analgin)	25	6	99.98	0.0486	15	5	100.17	0.0357
a. Analgin Tablet[2]	25	6	99.99	0.0095	15	5	99.75	0.0348
b. Novalgin Tablet[3]	25	6	100.40	0.0239	15	5	99.72	0.0400
4. Dimethyl amino-antipyrine (Amidopyrine) (Pure)	25	6	100.13	0.0219	15	5	100.28	0.0534
a. Esgipyrine[4]	25	6	100.25	0.0180	15	5	99.78	0.0538

* Average of 9 observations, SD Standard deviation;

1. CIPLA (India) Ltd.; 2. IDPL; 3. Hoechst Pharmaceutical Ltd.; 4. S. G. Chemicals & Pharmaceuticals (India) Ltd.

(Amidopyrine) in the pure form as well as in their pharmaceutical preparations at the mg level. The results are shown in Table 1.

The effect of different variables was studied in order to develop a simple and convenient procedure. In case of PHC it was found that 8M concentration of sulphuric acid provides suitable redox potential of $Fe(CN)_6^{3-}$. Similarly 4M HCl was needed for proper ionisation of NCS. A lower and higher concentration of acids gave inaccurate results. Addition of zinc sulphate (in case of PHC) shorten the reaction times through the formation of zinc potassium hexacyanoferrate(III) complex[17] $K_2Zn_3[Fe(CN)_6]_2$ which behaves as catalyst. While studying the effect of reaction time, it was observed that antipyrine reacts faster than other compounds both with PHC and NCS because of its simple structure as compared to Melubrin, Methyl Melubrin and Amidopyrine.

It was also noticed that the reactions with PHC are completed at 35°C while NCS reacts at room temperature (27°C). At higher temperature PHC and NCS decompose and give inaccurate results.

While studying stoichiometry it was noted that one mole of these medicinal compounds consume 6 moles of PHC. The final reaction products gave a positive test for the presence of the carboxylic group. Thus it is proposed that the methyl group at C_5 in antipyrine derivatives get oxidised to carboxylic group. This finds support from previous work[18].

In case of NCS it was observed that one mole of antipyrine consumes 6 moles of NCS where as Melubrin, Analgin and Amidopyrine consume 5 moles of NCS. In this reaction it is proposed that the compounds form hexachloro and pentachloro derivatives respectively.

Authors thank I.D.P.L. (India) for providing pure samples and showing their keen interest in the present research project.

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Ru(VI) catalysed oxidation of amines by alkaline hexacyanoferrate

(oxidation/amines/Ru(VI) catalysis/hexacyanoferrate)

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ABSTRACT Ru(VI) catalysed oxidation of benzyl amine and n-butyl amine by alkaline hexacyanoferrate(III) exhibits zero-order dependence in hexacyanoferrate(III) and first order in Ru(VI). Rate increases with an increase in [$\bar{O}$H]. A justifiable mechanism involving complex formation, supported by Michaelis and Menton plot due to deviation of linearity of reaction rate with amine at its higher concentration, is proposed.

Kinetic study of Ru(VI) catalysed oxidation of cyclic alcohols by hexacyanoferrate(III) in aqueous alkaline medium has been reported recently[1]. However, kinetic study of oxidation of amines is still lacking in the literature, and, therefore, the present study has been undertaken to determine the exact role of ruthenate ion Ru(VI) in the oxidation of benzylamine and n-butylamine by alkaline hexacyanoferrate(III) ions. Ru(VI) was prepared by the method reported elsewhere[1].

Reaction follows zero order kinetics in hexacyanoferrate(III) (Table 1). The value of (*-dc/dt*), *i.e.*, the decrease in rate (low extent) with decrease in concentration of hexacyanoferrate(III) may be due to the little contribution towards rate by the step (VI). Reaction follows first order kinetics in Ru(VI) (Fig. 1). In lower range of conentration of amine, linear proportionality with velocity is observed (Fig. 2) but at higher concentration veolcity attains a constant value. Reaction rate increases on increasing the [$\bar{O}$H] (Fig. 3).

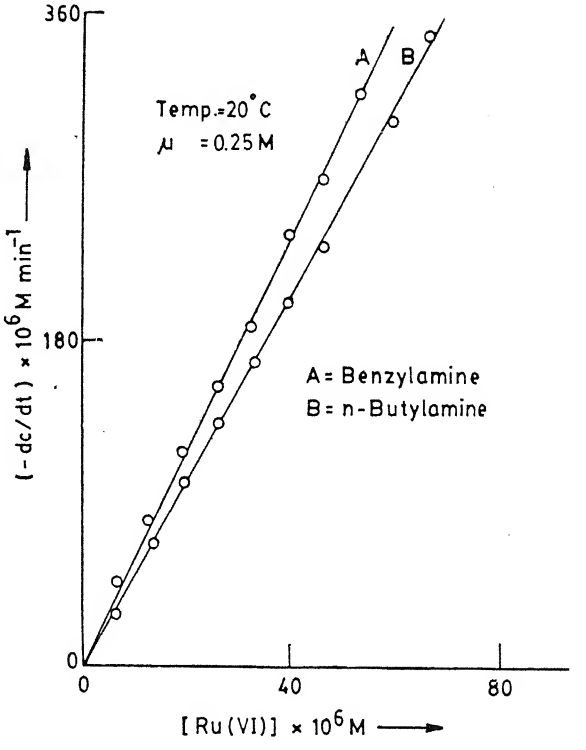


Fig. 1. Effect of variation of [Ru (VI)] on the reaction rate. [$\bar{O}$H] = 2.00 × 10^{-1}M. [$Fe(CN)_6^{-3}$] = 1.25 × 10^{-3}M
(A) [Benzylamine] = 1.25 × 10^{-2}M
(B) [n-Butylamine] = 6.25 × 10^{-2}M

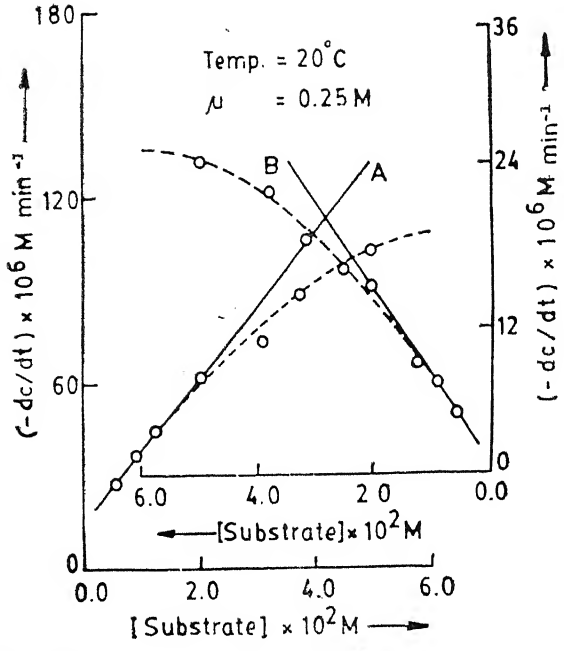


Fig. 2. Effect of variation of (Substrate) on the rate of reaction.
($\bar{O}H$) = 2.00 × 10⁻¹M, ($Fe(CN)_6^{-3}$] = 1.25 × 10⁻³M
(A) Benzylamine, [Ru(VI)] = 6.69 × 10⁻⁸M
(B) n-Butylamine, [Ru(VI)] = 13.40 × 10⁻⁸M

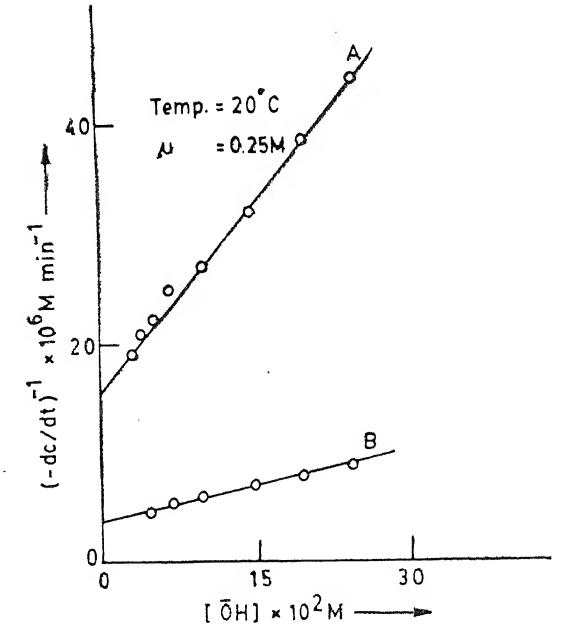
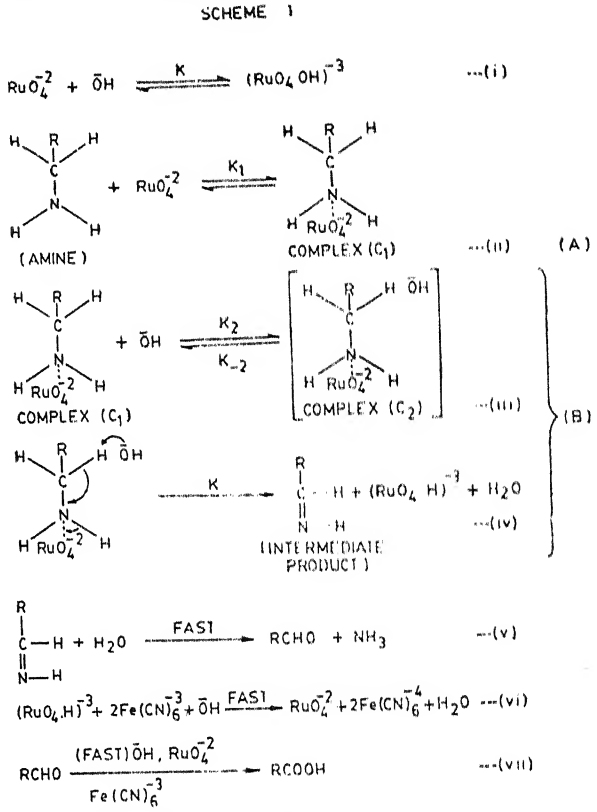


Fig. 3. Influence of ($\bar{O}H$) on the rate of reaction.
[$Fe(CN)_6^{-3}$] = 1.25 × 10⁻³M
(A) [Benzylamine] = 6.25 × 10⁻²M, [Ru(VI)] = 6.69 × 10⁻⁸M.
(B) [n-Butylamine] = 6.25 × 10⁻²M, [Ru(VI)] = 13.40 10⁻⁸M.

The confirmation of Ru(VI) as reactive species has already been highlighted in our recent publication.[1] Deviation from linear proportionality of reaction rate with respect to amine at its higher concentration supports the view that complex fromation[2] (Michaelis and Menton plot, Fig. 4) might take place with the substrate molecule.

On the basis of these results a probable mechanism for Ru(VI) catalysed oxidation of studied compounds is presented in Scheme 1.

SCHEME 1



where R is C_6H_5 or C_3H_7.

The step (B) is proposed on the basis of abstraction of α–protons in the oxidation of benzylamine by silver(II) picolinate in basic solution[3]. For confirmation, some test tube experiments have been performed with some non ionisable compounds (tertiary butanol) and it is observed that under identical conditions no appreciable oxidation occurs even in five hours.

TABLE 1

Dependence of reaction rate on hexacyanoferrate(III) at 20±1°C

$[\bar{O}H] = 2.00\times10^{-1}$M, $[(Ru(VI)] = 1.25\times10^{-8}$M, μ = 0.25 M								
[Benzyl amine]	$[K_3Fe(CN)_6]\times10^4$M	20.00	16.75	12.50	10.00	8.33	7.25	6.25
	$(-dc/dt)\times10^6$M min^{-1}	50.50	50.9	50.9	52.2	50.3	44.0	44.0
[n-Butyl amine]	$[K_3Fe(CN)_6]\times10^4$M	25.00	20.00	16.80	12.50	10.00	8.30	
	$(-dc/dt)\times10^6$ M min^{-1}	9.5	9.5	9.3	9.2	8.9	8.7	

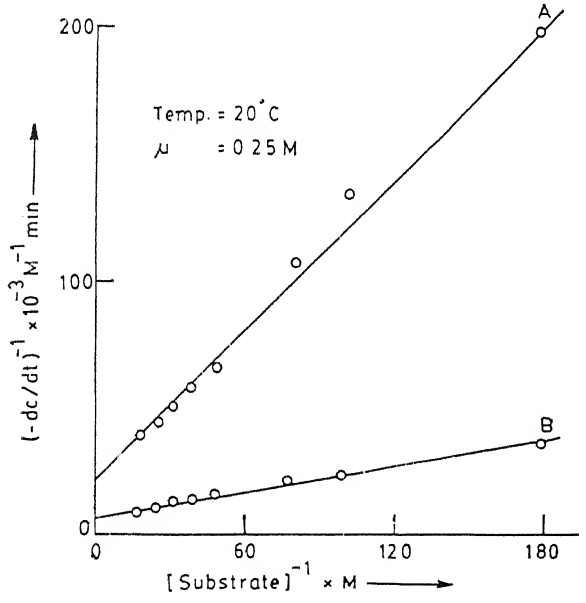


Fig. 4. Michael-Menten plot.
$[\bar{O}H] = 2.00\times10^{-1}$M, $[Fe(CN)_6^{-3}] = 1.25\times10^{-3}$M
(A) n Butylamine, $[Ru(VI)] = 13.40\times10^{-8}$M
(B) Benzylamine, $[Ru(VI)] = 6.69\times10^{-8}$M

From the scheme (1) the following rate expression is derived.

$$-\frac{d[(Fe(CN)_6^{-3}]}{dt} = \frac{2kk_2K_1[\bar{O}H][RCH_2NH_2][RuO_4^{-2}]_T}{(K+K_{-2})(1+K[\bar{O}H]+K_1[RCH_2NH_2])} \quad (1)$$

The rate law (1) successfully predicts the validity of experimental results and also accords well with the stoichiometry (1 ; 2) and values of various activation parameters (ΔE = 45.84 and 51.57 kJ/mole for benzylamine and n-butylamine respectively) (Table 2).

TABLE 2

Effect of temperature on rate of reaction

$[K_3Fe(CN)_6] = 1.25\times10^{-3}$M, $[\bar{O}H] = 2.00\times10^{-1}$M, μ = 0.25M				
[Substrate]	$(-dc/dt)\times10^6$M min^{-1}			
200×10^{-2} M	20°	25°	30°	35°
[Benzylamine]*	61.1	82.8	112.4	156.3
[n-Butylamine]**	15.1	19.8	26.4	35.0

*$[Ru(VI)] = 6.69\times10^{-8}$M, **$[Ru(VI)] = 13.4\times10^{-8}$M

Validity of results can also be verified by rearranging expression (1) and making an assumption that $1 \ll [RCH_2NH_2]$

$$\frac{1}{\text{Rate}} = \frac{K'}{2kk_2[\bar{O}H][RuO_4^{-2}]_T} + \frac{K'K}{2kk_2K_1[RCH_2NH_2][RuO_4^{-2}]_T} \quad (2)$$

where $K' = (k+k_{-2})$.

According to eqn. (2) a plot of $(-dc/dt)^{-1}$ *vs* [substrate]$^{-1}$ gives a straight line (Fig. 2) with a positive intercept on the rate^{-1} axis, and hence supports eqn. (ii). The intercept gives the value of

k. Similarly the plot of $(-dc/dt)^{-1}$ *vs* $[\bar{O}H]$ gives a straight line (Fig. 3) which supports the validity of eqn. (2).

A plot of $(-dc/dt)$ *vs* [Ru(VI)] also gives a straight line (Fig. 1) again supporting the rate law and hence the mechanism.

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Correlation between rate constant and empirical parameter of solvent polarity

(kinetics/empirical parameter/dielectric constant/rate constant/correlation analysis)

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ABSTRACT The rate constants of the reactions of propyl, propargyl and allyl bromide with thio-urea in various solvents as a single component solvent medium at 35°C are computed. A graph drawn between the logarithms of rate constants and Kirkwood parameter $(D-1)/(2D+1)$ for the above reactions exhibited a large scattering. Though only a poor relation is exhibited by a plot of $\log K$ *vs* E_T (Reichard's solvent parameter), it appears to be better than the Kirkwood plot. The positive slopes of the above plots indicate that the rate constant increases with increasing polarity of the solvent suggesting the formation of a polar transition state from non polar reactant molecules. Thus, it is evident that when pure solvents are used as a reaction medium the solvent polarity is a better parameter than the relative permitivity (dielectric constant).

The effect of the solvent on the rate of chemical reactions was first described in 1862 by Bertholot and Saint-Gilles in connection with their studies on the esterification of acetic acid with ethanol. Since then many workers studied the effect of solvent on different types of reactions and also established some qualitative and quantitative relations. The effect of solvent was explained in terms of solvent polarity such as dielectric constant. But the dielectric constant describes only the changes in the electric field intensity that occurs between the plates of a condenser when the latter is removed from vacuum and placed in a solvent. The dielectric constant, therefore, describes the ability of a solvent to separate electrical charges and orient its dipolar molecules. However, the total sum of the interactions between the solute and solvent molecules is much more extensive and complicated. In addition to the non-specific coulombic, directional, inductive and dispersion interactions, there are also specific hydrogen bond, electron pair donor (EPD)/electron pair acceptor (EPA) and solvophobic interactions. To circumvent such interactions, Reichardt[1] introduced empirical parameter of solvent polarity (E_T) based on the spectral absorption of a suitable standard dye (pyridiniophenolate). Keeping this in view we have studied the kinetics of alkyl bromides (propyl, propargyl and allyl bromides) with thiourea in various solvents as single component solvent medium at 35°C.

The neutral sulfur nucleophile thiourea B.D.H. was recrystallised twice from aqueous methanol and then used for the experiments. Allyl bromide (Koch-light), propargyl bromide (Fluka) and n-propyl bromide (B.D.H.,A.R.), were distilled and collected at their respective boiling points. The purity of the compounds was checked by estimating the bromide present in the compound by Volhard's method. All other solvents used were of A.R. grade and were purified by standard methods.

10 ml of nucleophile (0.02M) and 10 ml of alkyl bromide were placed separately in two boiling test tubes and placed in the thermostat for about 15 min to attain thermal equilibrium. The two solutions were mixed and the dry conductivity cell was placed in the reaction mixture. The progress of the reaction was followed by measuring the resistance of the reaction mixture using the Elico conductivity bridge type CM-82T at known intervals of time.

All the reactions studied between the sulfur nucleophile thiourea and alkyl bromides at 35°C followed second order kinetics proved by the linear plots of $R/R-R_\infty$ vs time, where R and R_∞ are resistances offered by the solution at time t and infinity. The rate constants calculated by the procedure of Frost and Pearson[2] were reproducible within the error ± 2–3% in all the cases. The rate constants are given in Table 1.

TABLE 1

Second order rate constants for reaction of n-propyl, propargyl and allyl bromide with thiourea in various solvents at 35°C

Solvent	D	E_T	$K \times 10^{-4}$ l^{-1} s^{-1}		
			allyl	propargyl	n-propyl
t-Butanol	12.2	43.9	151.4	52.48	3.46
2-Butanol	15.8	47.1	398.1	66.00	3.90
DMF	34.5	43.8	441.1	304.00	5.80
DMSO	48.9	45.0	460.5	320.00	6.90
CH_3CN	37.5	46.0	309.0	141.00	1.38
C_6H_5CN	25.2	42.9	141.3	40.00	1.29
Acetophenone	17.4	41.3	63.1	28.10	0.67
Sulfolane	44.0	44.0	126.0	70 80	2.63

To analyse the rate data, the following equation correlating the rate constant and dielectric constant for a dipole-dipole reaction

$$\ln k = \ln k^0 - \frac{N}{RT} \times \frac{D-1}{2D+1} \left(\frac{U_A}{r_A^3} + \frac{U_B}{r_B^3} - \frac{U^{\neq}}{r^{\neq 3}} \right)$$

is employed, where k is the rate constant in a medium of dielectric constant D, k^0 is the rate constant in a condensed medium of a unit dielectric constant, U_A, U_B and $U^{\neq}$ are the dipole moments of the two reactants and the transition state respectively and r_A, r_B and $r^{\neq}$ are the corresponding molecular radii. A graph is drawn between the logarithms of rate constants and Kirkwood parameter, $D-1/2D+1$ for the reactions of thiourea and substrates. The Fig. 1 shows the plot of a reaction between thiourea and allyl bromide. A large scattering is observed for this relation. Most probably the success of dielectric constant for a binary solvent system is due to the fact that the different interactions (as stated earlier) between the solvent pair and reactants remain constant, whereas only the dielectric constant is being continuously changed by the variation of the composition of reaction medium. When a single component (pure solvent) is used as a reaction medium the forces operating between the solvent and

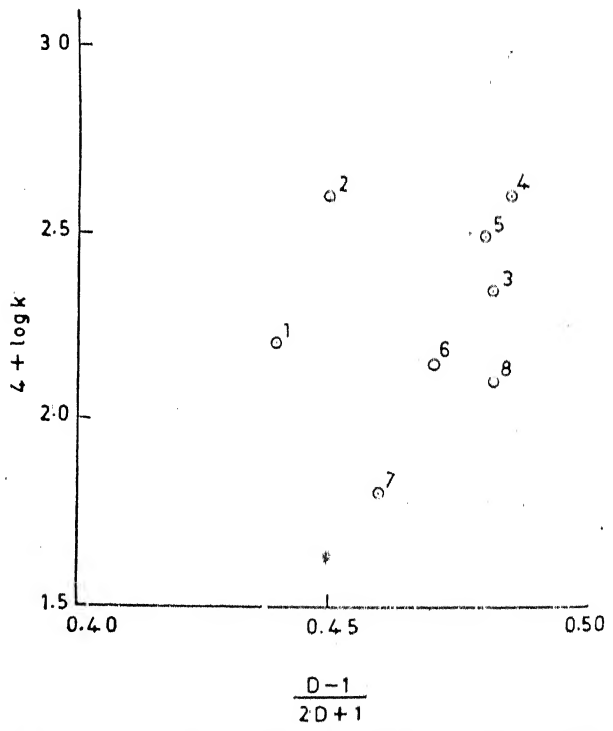

Fig. 1. Plot of a reaction between thiourea and allyl bromide

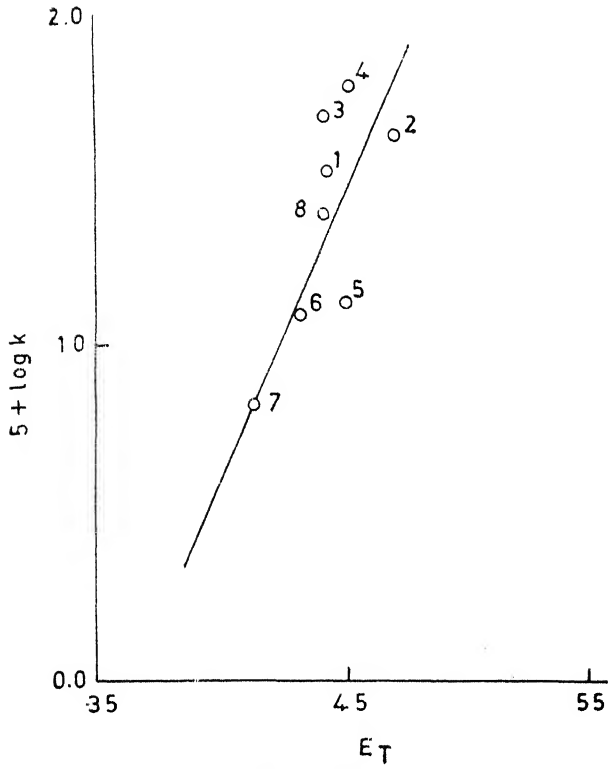

Fig. 2. Plot between E_T and $5+\log k$

solute are quite different. Under these circumstances the expectation of a correlation between the rate constant and Kirkwood parameter (dielectric constant) is unreasonable.

The failure of dielectric constant in correlation analysis led us to seek the help of solvent polarity, E_T. A plot of log K *vs* E_T values is a better correlation compared to the dielectric constant, though only a statistical correlation exists. Such a poor relation was also observed by Ballisteri and Maccarone[3]. The positive slope of the Fig. 2 indicates that the rate constant increases with increasing polarity of the solvent suggesting the formation of a polar transition state from non-polar reactant molecules. Thus, it is evident that when pure solvents are used as a reaction medium the solvent polarity (E_T) is a better parameter than the relative permitivity (dielectric constant).

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Kummer's function in fibre optic communication

(optical communication/fibre optics/optical waveguides)

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ABSTRACT Applications of Kummer's function in the study of fibre optic communication are pointed out. Numerical evaluation of this function for different parameters normally used for the fibre optic communication analysis purposes has been carried out through computer. A case for complex arguments has also been considered. The derivative has also been studied for both the real and complex arguments.

The regular increase in the use of transcendental function known as confluent hypergeometric function towards the analysis of physical and technical problems has allowed fibre optic physicists to use it for their analysis purposes. Becauses of three variables playing important role there, confluent hypergeometric functions provide an excellent set of functions for the exact solution of the wave equation particularly in graded index fibres[1]. Also, for the analysis of leaky modes and in particular for the study of attenuation and propagation of leaky modes in graded index fibres (both square law and distorted profile fibres), the complex nature of the function is to be studied[2,3]. The derivative with respect to some of the three variables for this function is also to be studied for both the real and complex arguments[3]. In this communication, computer programs have been developed and the numerical evaluation has been done for both the real and complex arguments. The derivative has also been computed. Importance of such calculations have been pointed out

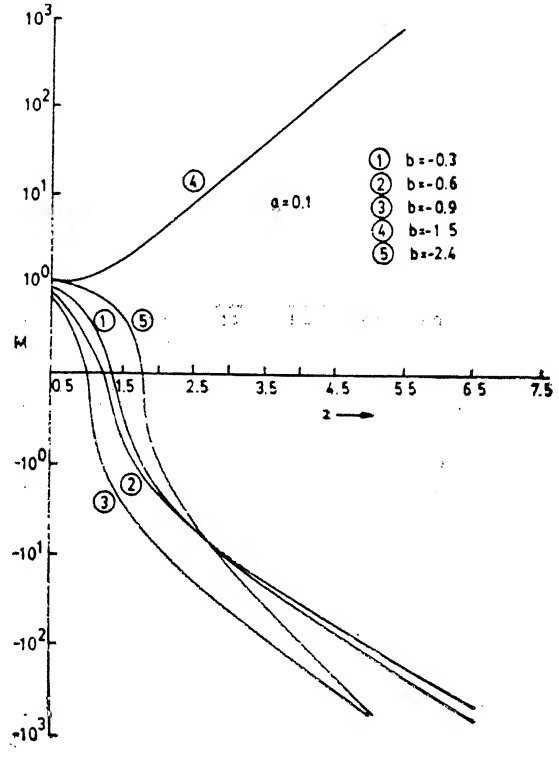

Fig. 1. Plot of Kummer function (M) as a function of z for values of $a = 0.1$, $b = -0.3$ (1), -0.6 (2), -0.9 (3), -1.5 (4), -2.4 (5).

The confluent hypergeometric equation also known as Kummer's equation is given by[4]

$$z\frac{d^2y}{dz^2} + (b-z)\frac{dy}{dz} - ay = 0 \qquad (1)$$

where a, b and z are real or complex as the case may be. Eqn. (1) has the regular singularity at $z=0$ and irregular essential singularity at $z=\infty$. A solution of eqn. (1) is the Kummer function represented by ${}_1E_1(a, b, z)$ or $M(a, b, z)$ given by

$${}_1F_1(a, b, z) = M(a, b, z) = \sum_{n=0}^{\infty} \frac{\Gamma(a+n)}{\Gamma(a)} \cdot \frac{\Gamma(b)}{\Gamma(b+n)} \cdot \frac{z^n}{n!} \qquad (2)$$

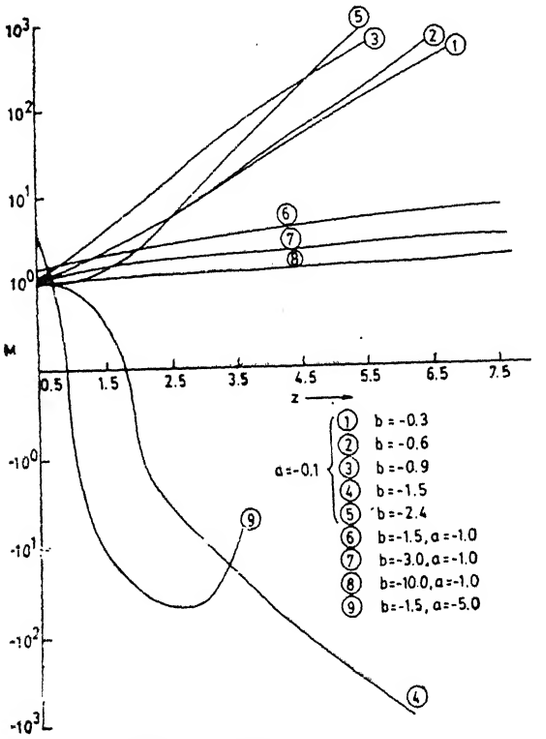

Fig. 2. Plot of M as a function of z for combinations of (1) ($a = -0.1, b = -0.3$), (2) ($a = -0.1, b = -0.6$), (3) ($a = -0.1, b = -0.9$), (4) ($a = -0.1, b = -1.5$), (5) ($a = -0.1, b = -2.4$), (6) ($a = -1.0, b = -1.5$), (7) ($a = -1.0, b = -3.0$), (8) ($a = -1.0, b = -10.0$), (9) ($a = -5.0, b = -1.5$)

The corresponding confluent hypergeometric equation for graded index optical fibre is given by

$$\frac{d^2f}{dr^2} + \frac{1}{r}\frac{df}{dr} + \left[K_0^2 n^2(r) - \frac{l^2}{r^2} - \beta^2 \right] f(r) = 0 \qquad (3)$$

Eqn. (3) was derived by solving Maxwell's equations to give wave equations in cylindrical coordinates (r, ϕ, z) with the case of a parabolic refractive index profile. Field in the core would be given by $\frac{1}{r} M_{k,l}\left(\frac{r}{w_0^2}\right) e^{\pm i l \phi}$ where $M_{k,l}$ are Whittaker function and other terms have their usual meanings in the optical fibre. The eigenvalue equation that determines the cut off frequencies in a graded index fibre is given by

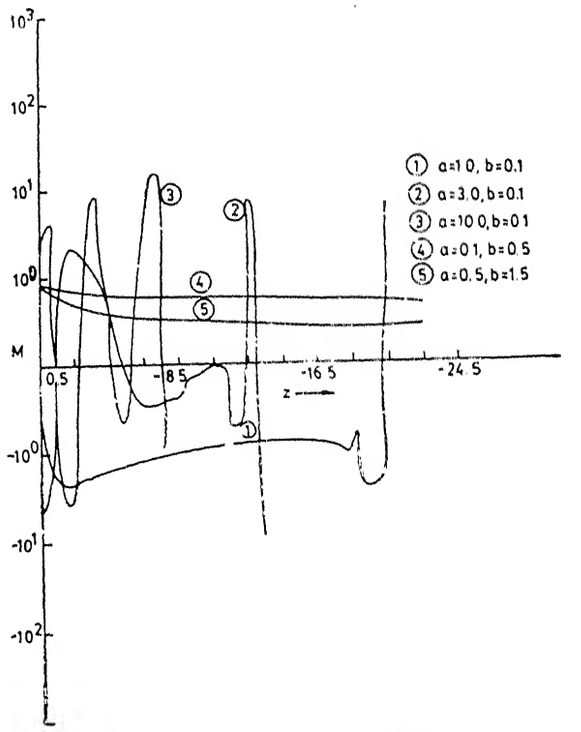

Fig. 3. Plot of M as a function of z (negative values of z) for combinations of (1) ($a = 1.0$, $b = 0.1$), (2) ($a = 3.0$ $b = 0.1$), (3) ($a = 10.0$, $b = 0.1$), (4) ($a = 0.1, b = 0.5$), (5) ($a = 0.5, b = 1.5$).

$$\left[1+\frac{2l-2}{V_c}\right] M\left(\frac{l}{2}-\frac{V_c}{4}, l, V_c\right)$$

$$=\left[1+\frac{V_c}{2l}\right] M\left(\frac{l}{2}-\frac{V_c}{4}, l+1, V_c\right) \quad (4)$$

where M represents the Kummer function and other terms have their usual meanings in fibre optic communication. For an optical fibre case the arguments in Kummer function $M(a, b, z)$ have different combinations. In particular, for the case of leaky modes a and b are complex quantities. We have computed on a DEC 10 machine $M(a,b,z)$ for various combinations of a, b, z. The results are plotted in Figs. (1-4). For the case of complex argument, the results are shown in Fig 5. Here both the real and imaginary values of the computed function are shown in the same curve. The combinations (a_r, b_r, z_r) give the real value of M whereas the combinations (a_i, b_i, z_i) give the imaginary part. It should be noted that here in the plots z_r, z_i, a_r and a_i follow the same x axis and a point there indicates values for all of them. The derivative of M with respect to one of its arguments particularly that of a is also required for the calculation of leaky modes. We have also computed it. The formulae to evaluate it have been derived and are given by

$$DM = \frac{\partial M(a, b, z)}{\partial a} = \sum_{n=1}^{\infty} \frac{a(a+1)\ a+2\ldots(a+n-1)}{b(b+1)(b+2)\ldots(b+n-1)} \sum_{m=1}^{n} \frac{1}{a+m-1} \cdot \frac{z^n}{n!} \quad (5)$$

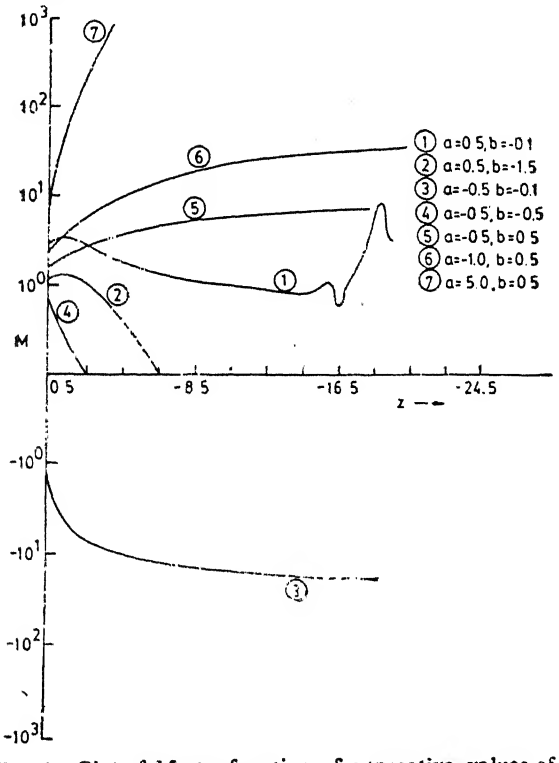

Fig. 4. Plot of M as a function of z (negative values of z) for combinations of (1) $(a = 0.5, b = 0.1)$, (2) $(a = 0.5, b = -1.5)$, (3) $(a = -0.5, b = -0.1)$, (4) $(a = -0.5, b = -0.5)$, (5) $(a = -0.5, b = 0.5)$, (6) $(a = -1.0, b = 0.5)$, (7) $(a = 5.0, b = 0.5)$.

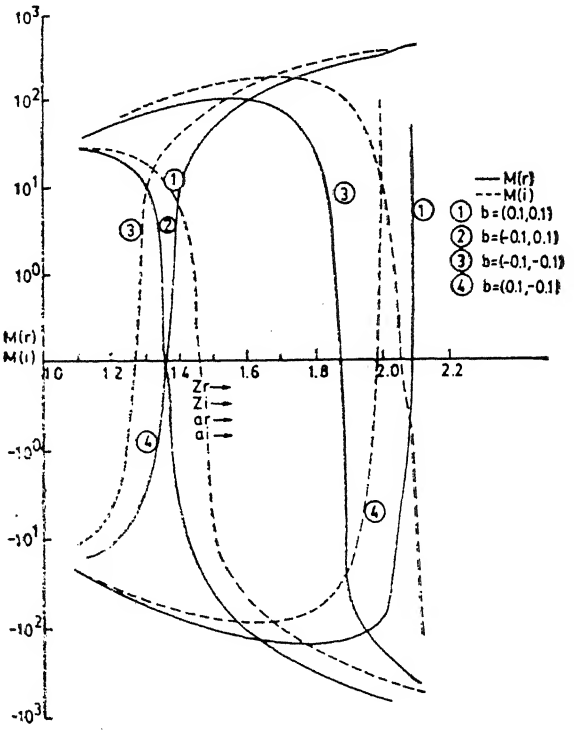

Fig. 5. Plot of real and imaginary values of M (Mr and Mi resepectively) for

(1) b = (0.1, 0.1), (2) b = (-0.1, 0.1), (3) b = (-0.1, 0.1) and (4) b = (0.1, -0.1)

Fig. 6 gives the plot of real and imaginary component of the derivative for the real and imaginary components of the argument. The above evaluation of Kummer function and its derivative for various combinations of its arguments (both real and imaginary) essentially give the eigenvalues (both real and imaginary) as a function of cut off frequencies of various propagating modes in the graded index optical fibre. These have been utilised to study leaky modes and their attenuation etc. and are discussed elsewhere[5,6].

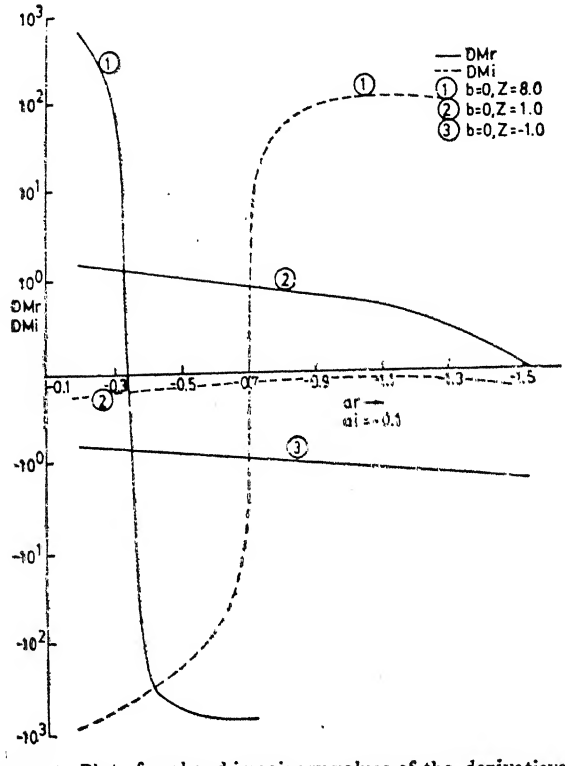

Fig. 6. Plot of real and imaginary values of the derivatievs (*DMr* and *DMi* respectively) for (1) b = 0, Z = 8.0, (2) b = 0, Z = 1.0, (3) b = 0, Z = −1.0

The computations on DEC 10 machine were done at the Institute of Advanced Studies, Australian National University (Department of Applied Mathematics), Canberra, Australia.

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Nat. Acad. Sci. Letters, Vol. 9, No. 8, 1986

Changes in haemolymph protein pattern of male and female adult *Spodoptera litura* Fab.

(haemolymph protein(HP)/oviposition/*Spodoptera litura*)

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ABSTRACT The communication describes the differences observed between two sexes of *S. litura* Fab. adult. A maximum of 11 protein bands were separated. Fractions 2, 4 and 19 appear to be major protein bands. The reduction in fractions 2 and 4 on 2nd day male and fraction 19 in female after oviposition suggests their utilization in the reproductive process.

Although considerable work on the haemolymph protein of adult insects in different orders has been done[1-8] but so far no systematic work is reported on day-to-day changes in HP pattern of adult moth. Keeping this in view the present work was undertaken to elucidate the physio-biochemical balance between protein types and amounts in its relation to uptake of proteins in the egg during the time of oogenesis.

The culture of *Spodoptera litura* Fab. was initiated with a single pair obtained from the field. The larvae were raised in Zoology Department, Allahabad University on castor leaves at 27±2°C.

Haemolymph samples of each sex were collected from male and female into specimen tubes containing phenyl-thio-urea (tyrosinase inhibitor). After centrifugation of samples at 3000 rpm for 5 minutes, they were applied for polyacrylamide gel-electrophoresis as described by Davis[9] and Ornstein[10]. The scanning of tube gels was carried out by Gilford UV-visible spectrophotometer Model 250 at 560nm and absorbance has been recorded on Honeywell charts 680030-075 with the help of Gilford heat sensitive recorder. The identification of protein bands and their relative mobility values are based on the densitometric recordings.

The electrophoretic patterns of haemolymph protein of males and females are presented in Fig. 1. A maximum of 11 protein bands could be separated from these haemolymph samples. Fractions 2,4 and 19 appear to be major protein bands. Other HP fractions vary in number and concentration since they occur only as weak bands or traces. It is of interest to note that most of the oocyte maturation is completed at the time of adult emergence and the mating takes place on 2nd day after the emergence and the copulation continues till the end of the 3rd day after which the egg laying takes place on the 4th day. The reduction in fraction 2 and 4 on 2nd day in male and fraction 19 in female after oviposition (*i.e.* after 4th day) clearly suggests their utilization in the reproductive process *viz.*, formation of seminal fluid in the males and maturation of oocytes in the female.

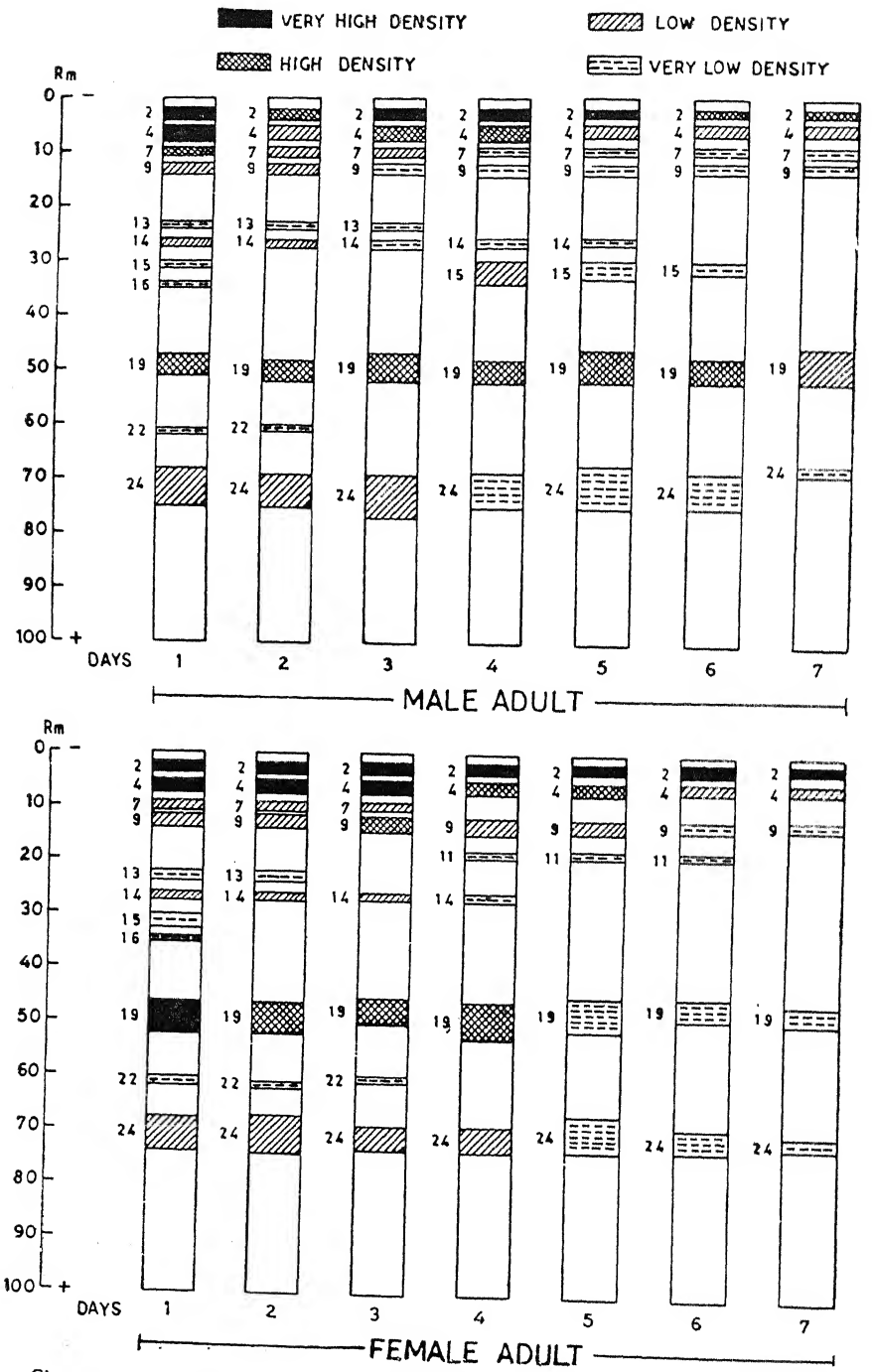

Fig. 1. Changes in haemolymph protein pattern of male and female adult *Spodoptera litura* Fab.

Although quantitative estimations could not be performed, the concentration and the width of the protein band clearly indicate that there is a rise in HP concentration during first day after emergence, after which there is a decline on the 2nd day followed by a slight build up of protein bands again on 3rd day and then their depletion thereafter. The rise of HP concentration after emergence and fall after oviposition was also noticed in *Phormia regina*[11] and *Schistocerca gregaria*[2]. In the house cricket *Acheta domesticus*, Nowosielski and Patton[3] found that the HP concentration decreases gradually during the course of adult life The decrease in the HP of adult females may be used to follow the course of deposition in the ripenning eggs. Telfer[12] found that some HP enters the oocytes in *Hyalophora cecropia*. This is also indicated in *Schistocerca gregaria*[6]. The concentration of each fraction decreases with the lapse of time after emergence which may also be due to the process of ageing.

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Induction of lethal mutations in the fish, *Oreochromis mossambicus* by an insecticide, Rogor 30E and the method of its detection

(lethal mutations/Rogor 30E/fish)

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ABSTRACT The mouth brooding habit of females of the exotic fresh water tilapian species, *Oreochromis mossambicus* has been exploited in assessing the lethal mutations induced by the organophosphorus inseticide, Rogor 30E. Sexually mature males injected intraperitoneally with Rogor solution were allowed to mate with different sets of virgin females consecutively for four weeks and the frequency of unfertilized eggs and unhatched embryos was found each week to be higher than that of control.

Among various mutagenicity testing protocols, assay of lethal mutation, discovered as early as 1935 in mice as a model[1,2], has been considered to be a very crucial test. Since then the method has further been improved to assay pre- and post-implantation loss during gestation[3] and after parturition at the end of the weaning period[4], sensitivity of different stages of spermatogenesis[5] etc. On the other hand, among various species of fishes deployed as models for testing mutagenic potentialities of odd agents[6-8], 'madeka'– *Orizias latipes*[9] and the tilapian species, *Oreochromis mossambicus*[8,10] were found very useful for assaying lethal mutations for having various advantages[8,9]. However, among these two species, tilapia has been preferred[8,10] since mass culture of this edible species with a view to supplementing animal proteins to many tropical, subtropical and developing countries[11,12] constitutes an international pragramme and they have been popularly called as 'Aquatic Chicken'[13]. The clastogenic potentiality of the insecticide, Rogor 30E has been shown by micronucleus test[14] while the present study was undertaken to test its mutagenic potentiality.

The tilapian species introduced as *Tilapia mossambicus* had undergone taxonomic revision and is now known as *Oreochromis mossambicus* on the basis of its fresh water habitat and mouth brooding habit of females[13]. Manna[6-8] advocated tilapia as a useful model for genotoxic assays for reasons like small size, suitable karyotype with a pair of conspicuously large marker chromosomes, early sexual maturity with sexual dimorphism, can breed thrice a year to which the mouth brooding habit of female parent is of great advantage in the study of lethal mutation because after mating the eggs laid by the female parent are taken into her buccal cavity for brooding. This makes mouth swollen and can be detected externally without disturbing the female. This mouth brooding habit is a great help in the assessment of lethal effect, if any, because by natural instinct the female parent takes all the eggs laid by her into her mouth cavity. If the tail of such a

female is held by hand so that the mouth points downwards after 48 h of incubation, she releases all the eggs and embryos from her buccal cavity which on examination may be sorted as : (i) unfertilized eggs appearing whitish with no signs of developing embryos, (ii) dead unhatched embryos seen through the cover of egg membrane and (iii) living embryos with yolk.

In the present study 11 mature male specimens weighing individually between 20g and 25g were intraperitoneally injected with Rogor solution (1.2 ppm, LC50 at 96 h) at the rate of 1 ml per 100g body weight. Each treated male was then left in a vat containing 4 mature virgin females at a time. The vats were inspected daily and if any female was found to have eggs in her mouth cavity, she was very gently transferred to another aquarium and a fresh mature virgin female was put in her place. In this way the same treated male was allowed to mate continuously for four weeks with different set of four virgin females. Each isolated female was left undisturbed after transfer for 48 h and then eggs released from her mouth were collected in a petridish containing tap water by the method described above. The eggs collected in the petridish were left at room temperature for another 72 h and during the period the number of unfertilized eggs, unhatched and hatched embryos per female were determined. Parallaly normally mature males injected with the same dose of distilled water were allowed to mate with virgin females. The data of eggs and embryos per female from these served as control. The average number of eggs, fertilized and unfertilized and unhatched embryos per female in four weeks mating programme of control and treated series was determined, and the lethal effect, mutagenic index, etc. were calculated (Table 1).

The present data show that the average number of eggs per female in treated series was more variable than that of control (Table 1). The average number of unfertilized eggs or unhatched embryos was significantly higher than that of control indicating the lethal mutation induced by the treatment of the pesticide Rogor to male parents. Lastly the mutage-

TABLE 1

Lethal effect and mutagenic index in F_1 generation of control Rogor 30E treated male tilapia mated to different set of virgin females in different weeks

Mat wk	Sr	No. of Fem	Total No of eggs	Average number per mother: No of eggs	No of fertilized eggs	No of unfertilized eggs	No of unhatched embryos	Mutagenic index
1st	Cont.	7	765	109.28±15.99	108.42±16.12	1.00±0.36	0.66±0.21	0.52
	Treat	3	486	162.00± 5.68	159.66± 6.00	2.33±0.33 d	13.00±2.80 c	8.14
2nd	Cont.	11	1075	97.72± 8.04	96.81± 7.81	0.91±0.28	0.54±0.20	0.56
	Treat	11	1412	128.36± 9.27	115.18± 7.71	13.18±2.85 a	6.91±1.28 a	5.92
3rd	Cont.	11	1053	95.73± 3.63	94.8 ± 3.14	0.91±0.72	1.09±0.51	1.13
	Treat	11	1028	93.45± 9.01	90.54± 9.45	2.91±1.24 d	5.82±1.16 b	5.47
4th	Cont.	11	1278	116.18±10.30	114.72±10.08	1.45±0.36	1.00±0.19	0.87
	Treat	11	1263	114.85± 9.46	104.45± 7.83	10.36±2.40 b	12.82±3.55 b	11.16
TOTAL	Cont.	40	4171	104.72± 9.49	103.69± 9.29	1.07±0.43	0.82±.28	1.95
	Treat	36	4189	124.66± 8.35	117.45± 7.75	7.19±1.70	9.64±2.20	6.33

Significance level : a = $P<0.001$; b = $P<0.01$; c = $P<0.05$ and d = $P>0.05$

nic index in treated series of different weeks varied more than that of control but it was always much higher than control. Since the time-table for spermatogenesis and spermiogenesis in male tilapia is not known, it is not certain whether there is any stage sensitivity of the Rogor effect as in mice[5]. Further studies are in progress.

Geateful acknowledgement is made to the University Grants Commission for providing financial assistance to carry out the work.

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Hymenolepis nana infection in mice : Effect of hydrocortisone acetate on the retention and development of worms up to the adult stage

(hydrocortisone acetate effect/mice/worm infection/*Hymenolepis nana*)

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ABSTRACT Treatment of female Swiss albino mice with HcA brought about immunosuppression which caused increased retention of *H. nana* at cysticerocid stage (96 hours) in comparison to adults at 21 days after infection. Immunosuppressed mice infected with a single dose of 500 eggs were endowed increased retention capacity. However, treatment did not promote worm development.

There are several ways to study immune response in a host and one of them is by employing an immunosuppressive agent like hydrocortisone acetate which causes physiological disturbances. *Hymenolepis nana*, the human dwarf tapeworm, also occurring in rodents elicits a highly immunogenic response due to an intravillous cysticercoid stage in comparison to the lumenal stage[1]. Weinmann and Rothman[2] reported that resistance acquired by mouse to *H. nana* following a primary egg infection is cortisone sensitive both during induction (cysticercoid) as well as the establishment (adult) phases and, thus, the acquired resistance is partially suppressed by cortisone[3]. On the other hand, cortisone could also bring about superinfection of worms in gut and metastasis of cysticercoids in the mesenteric lymph nodes of liver of mice[4]. Such studies on the interaction between *H. nana* and the immunosuppressed state of mice help us not only to understand the underlying immune mechanism but also suggest ways for the prevention and deestablishment of such infection under variable conditions. Thus, an attempt has been made to study the effect of hydrocortisone acetate on the retention capacity, development and establishment of cysticercoids initially and adults of *H. nana* subsequently in treated mice.

Four groups, A, a. B and b of female Swiss albino mice 20 in each weighing 25 g were used. Group A was divided into two subgroups, A_1 and A_2, group B into subgroups, B_1 and B_2, group a into a_1 and a_2 and group b into subgroups, b_1 and b_2. Mature viable *H. nana* were collected and counted according to the hook-worm egg dilution method described by Heynemon[1]. Groups A_1, A_2, B_1 and B_2 were injected with hydrocortisone acetate (Roussal) subcutaneously (50 mg/kg body wt.) daily for 6 days and ware infected per os with a single dose of 500 (Groups A_1 and A_2) and 2,500 (Groups B_1 and B_2) *H. nana* eggs on day 4 of the drug treatment. Mice of groups a_1, a_2, b_1 and b_2 were taken as controls and received only 500 (groups a_1, and a_2) and 2,500 (groups b_1 and b_2) *H. nana* eggs. Degree of immunity was assessed by counting cysticercoids (groups A_1, a_1, B_1 and b_1) on the 4th day (after 96 hours) and mature adults (groups A_2 a_2, B_2 and b_2) on 21st day after infection. Table 1

gives the experimental protocol of HcA treatment and infection in animals.

TABLE 1

Experimental schedule of HcA treatment (A_1, A_2, B_1 and B_2), infection with single low (A_1, A_2, a_1 and a_2 – 500) and high (B_1, B_2, b_1 and b_2–2500) doses of *H. nana* eggs to mice groups and subsequent recovery of cysticercoids and adults.

Days	Experimental groups A_1 & A_2	B_1 & B_2	Control groups a_1 & a_2	b_1 & b_2
0	HcA treatment starts 50 mg/kg	HcA treatment starts 50 mg/kg	–	–
1	,,	,,	–	–
2	,,	,,	–	–
3	,, +500	,, +2500	500	2500
4	,,	,,	–	–
5	,,	,,	–	–
7	Cysticercoid counts in the four groups A_1, a_1, B_1 & b_1.		–	
21	Adult counts in the four groups – A_2, a_2, B_2 & b_2.			

Results are presented in Table 2 and 3 and their statistical analysis are presented in Table 4. Experimental (treated) mice of groups A_1 and B_1 retained significantly greater percentage of cysticercoids (12.4% and 6.55% respectively) in comparison to their counterpart controls a_1 and b_1 (6.6% and 1.76% respectively).

TABLE 2

Cysticercoid counts at 96 hours from mice of experimental (A_1 and B_1 treated with hydrocortisone acetate for 6 days) and control (a_1 and b_1 untreated) groups infected with 500 (A_1 and a_1) and 2500 (B_1 and b_1) viable eggs of *H. nana*.

Group	Dose of infection	Range	Average/mouse
A_1	500	37–147	62 (12.4%)
a_1	500	31–42	33 (6.6%)
B_1	2500	150–192	163 (6.55%)
b_1	2500	39–47	44 (1.76%)

The average number of adults counted from group A_2 was 39.5 (7.9%) of which 0.3% were large 0.4% medium and 7.2% small, whereas in the counterpart controls, their average number was 25.5 (5.1%), of which 0.4% were large, 2.5% medium and 2.2% small. In group B_2, the mean number of adults was 51.5 (2.16%) of which 1.32% were large, 0.62% were medium and 0.22% small whereas control mice harboured an average of 15 (0.6%) of which 0.28% were large, 0.2% medium and 0.12% small. When these results were submitted to Students t-test, it was found that the retention of worm burden in the experimental groups was highly significant in comparison to that of controls (Table 3).

TABLE 3

Adult counts at 21st day from mice of experimental (A_2 and B_2 treated with hydrocortisone acetate for 6 days) and control (a_2 and b_2 untreated) groups infected with 500 (A_2 and a_2) and 2500 (B_2 and b_2) viable eggs of *H. nana*.

Group	Dose of infection	Adult worms recovered (average No /mouse) Large 60 mm	Medium 30-59 mm	Small 1-29 mm	Total (mean)
A_2	500	1.5 (0.3%)	2.0 (0.4%)	36.0 (7.2%)	39.5 (7.9%)
a_2	500	2.0 (0.4%)	12.5 (2.5%)	11.0 (2.2%)	25.5 (5.1%)
B_2	2500	33.0 (1.32%)	13.0 (0.62%)	5.5 (0.22%)	51.5 (2.16%)
b_2	2500	7.0 (0.28%)	5.0 (0.2%)	3.0 (0.12%)	15.0 (0.6%)

TABLE 4

Statistical analysis (differences between means, Student's t-test) among the various experimental (A_1, A_2, B_1 and B_2) and control (a_1, a_2, b_1 and b_2) groups of mice treated with HcA and infected with *H. nana*.

Group	t-value	Group	t-value
A_1 *vs.* a_1	3.021*	B_2 *vs.* b_2	5.137*
B_1 *vs.* b_1	45.642*	A_2 *vs.* B_2	3.452*
A_1 *vs.* B_1	9.665*	A_1 *vs.* A_2	2.317*
A_2 *vs.* a_2	6.624*	B_1 *vs.* B_2	21.439*

P-value at 5% level of significance is 2.101. *Statistically significant value.

As a natural occurrence, a small percentage of worms in both the experimental (approximately 3-5%) and control (approximately 1-2%) groups were

expelled during 4–21 days period when the cysticercoids developed into adults. Even taking this into account, it was found that the number of cysticercoids developed and retained were far greater at 96 hours than the adults which could establish themselves at 21st day indicating the absolute influence of HcA at the cysticercoid level in the experimental groups. In experimental group A_2 with 500 eggs, cortisone while influencing the capacity of animals to retain a larger percentage of worms, has not in anyway promoted their development and growth with the result that majority of the worms in the population retained, consists of small sized worms. However, in experimental group B_2 (with 2500 eggs), the results are rather contradictory with a greater percentage of worms in the large-sized category. This may be due to the fact that though cortisone has been recognised to promote growth, certain, stressful conditions within the host (for example, heavy worm burden or hormonal imbalance) may result in a greater number of worms to be favoured with growth and development as pointed out by Moss[5] who reported the combined action of cortisone on mice as an immunosuppressant as well as a growth promoter.

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General Secretary
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